Title

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Journal

Journal of the National Cancer Institute, 102(19)

ISSN

0027-8874

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Publication Date

2010-10-06

DOI: 10.1093/jnci/djq325
Advance Access publication on August 26, 2010.

BRIEF COMMUNICATION

Ornithine Decarboxylase-1 Polymorphism, Chemoprevention With Eflornithine and Sulindac, and Outcomes Among Colorectal Adenoma Patients

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Manuscript received December 10, 2009; revised July 22, 2010; accepted July 26, 2010

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The ornithine decarboxylase-1 (ODC1) polymorphism at position +316 affects binding by transcriptional activators and repressors and modulates the risk of metachronous colorectal adenomas, particularly in association with aspirin use. We investigated the effects of ODC1 after treatment with difluoromethylornithine (eflornithine)/sulindac or placebo. Two hundred twenty-eight colorectal adenoma patients in a randomized phase III trial were genotyped for ODC1. We used Wilcoxon rank sums tests on non-normally distributed continuous variables across two genotype groups, χ^2 or Fisher exact test to assess the association between baseline categorical variables and genotype group, and log binomial regression for the primary (adenoma recurrence) and secondary outcomes (tissue polyamine response, cardiovascular toxicity, gastrointestinal toxicity, and ototoxicity). All statistical tests were two-sided. In binomial regression models with variables age, sex, race, aspirin use, treatment, and ODC1 genotype, treatment was the only statistically significant factor associated with differences in adenoma recurrence or tissue polyamine response. A statistically significant interaction was detected between ODC1 genotype and treatment with respect to adenoma recurrence (placebo group: GG, 50%, AA/GA: 34%; treatment group: GG, 11%, AA/GA, 21%; $P_{interaction}$ = .038). Excess ototoxicity was observed among ODC1 AA patients receiving treatment, but the interaction of genotype and treatment on ototoxicity was not statistically significant (P = .45).

J Natl Cancer Inst 2010;102:1513–1516

A single-nucleotide polymorphism in intron 1 of human ornithine decarboxylase-1 (*ODC1*) has functional consequences for binding by E-box activators and repressors (1,2) and has been investigated as a marker for colorectal adenoma risk (3–5). The reported minor A allele frequency is approximately 25%, and despite differences across race or ethnicity, *ODC1* genotype distribution is in Hardy–Weinberg equilibrium within each race (white, black, Hispanic, Asian) (2,6). Individuals homozygous for the *ODC1* minor A allele have reduced risk of adenoma recurrence compared with those with the major G allele (3,5). Furthermore, the *ODC1* A allele (AA or GA genotype but not GG genotype) and reported aspirin use have been associated with reduced colon polyp recurrence (3–5) and with a statistically significant 50% reduced risk of advanced adenomas (4). Recently, we demonstrated the efficacy of a polyamine inhibitory combination of long-term daily oral D,L-α-difluoromethylornithine and sulindac among colorectal adenoma patients (7); however, treatment was associated with modest subclinical ototoxicity (8) and a greater number of cardiovascular events among patients with high baseline cardiovascular risk (9). Here, we investigate whether the *ODC1* genotype differentially affects adenoma recurrence, tissue polyamine responses, or toxicity profiles after eflornithine and sulindac treatment compared with placebo.

We analyzed patient data from the multicenter phase III colon adenoma prevention trial (ClinicalTrials.gov identifiers NCT00005882, NCT00118365) (7). Three hundred seventy-five patients were enrolled, and the study was halted by the Data Safety Monitoring Board after 267 patients completed end-of-study colonoscopies (because of the study meeting its efficacy endpoints). The Data Safety Monitoring Board monitored all safety and efficacy endpoints. Blood specimens were collected from 228 consenting study patients for genotyping analysis after November 2002 (including 159 of the 246 patients randomly assigned before this date, and 69 of the 129 patients randomly assigned after this date) when the protocol was modified in light of data demonstrating the importance of the *ODC1* single-nucleotide polymorphism (3). *ODC1* (rs2302615) genotyping was conducted on patient-derived genomic DNA using allele-specific TaqMan probes as described previously (1). Rectal tissue polyamine content was determined as described previously (10,11) using three of the eight randomly selected rectal mucosal biopsy specimens. Tissue polyamine response was performed for response values ranging from 25% to 45%.

The *ODC1* genotype was analyzed under a dominant model: AA/GA vs GG patients. Wilcoxon rank sums tests were performed on non-normally distributed continuous variables across two genotype groups. The χ^2 or Fisher exact test was used to assess the association between baseline categorical variables and genotype group. Log binomial regression was performed on the primary outcome (adenoma recurrence), with the variables treatment group, age, sex, race (white vs other), aspirin use, *ODC1* genotype (in the dominant model), and a term representing the treatment by genotype interaction. For secondary outcomes (rectal tissue polyamine response, cardiovascular toxicity, ototoxicity), the effects of treatment group, genotype, and interaction between treatment and genotype were examined using full log binomial models. For the analysis of the secondary outcome gastrointestinal toxicity, because of imbalances in the distribution by gender and race, the effects of treatment group,

CONTEXTS AND CAVEATS

Prior knowledge

Individuals homozygous for the minor A allele of the ornithine decarboxylase-1 (*ODC1*) gene have reduced risk of colorectal adenoma recurrence compared with those with the major G allele.

Study design

The associations among *ODC1* genotype, tissue polyamine responses, and toxicity after eflornithine and sulindac treatment compared with placebo were investigated with patient data from a multicenter phase III clinical trial of colorectal adenoma prevention.

Contribution

A statistically significant interaction between *ODC1* genotype and treatment with respect to adenoma recurrence was found. Individuals homozygous for the G allele had lower risk of adenoma recurrence after eflornithine and sulindac treatment than those homozygous or heterozygous for the A allele. *ODC1* AA patients receiving treatment had excess ototoxcity, but the interaction between genotype and treatment on ototoxicity was not statistically significant.

Implications

Although the minor A allele protects individuals against colorectal adenoma recurrence, especially in association with aspirin use, carrying two copies of the G allele reduces the risk of recurrence after treatment with eflornithine and sulindac.

Limitations

Study limitations include small sample size and a lack of balance in baseline characteristics across *ODC1* genotype groups. Sulindac and its metabolites also have polyamine inhibitory properties and other antineoplastic mechanisms, such as cyclooxygenase inhibition and β-catenin degradation, which could account for the findings.

From the Editors

Table 1. Clinical characteristics of all subjects at baseline by *ODC1* genotype*

Characteristics	*ODC1* AA/GA genotype (n = 102)	*ODC1* GG genotype (n = 126)	*P*†
Mean age ± SD, y	60.2 ± 8.4	62.6 ± 8.7	.024‡
Sex, No. (%)			
Male	77 (75)	96 (76)	.90
Female	25 (25)	30 (24)	
Race, No. (%)			
White	84 (82)	107 (85)	.007§
Black	3 (3)	4 (3)	
Hispanic	4 (4)	12 (10)	
Asian	9 (9)	1 (1)	
Other	2 (2)	2 (2)	
Treatment group, No. (%)			
Eflornithine + sulindac	46 (45)	71 (56)	.09
Placebo	56 (55)	55 (44)	
Low-dose aspirin use, No. (%)			
Yes	44 (43)	54 (43)	.97
No	58 (57)	72 (57)	
Median No. (minimum–maximum)	2.00 (1–11)	2.00 (1–16)	.41‡
Location of largest prior polyp, No. (%)			
Rectum	26 (25)	23 (18)	.19
Colon	76 (75)	103 (82)	
Prior polyp histology, No. (%)			
Tubular	76 (75)	99 (79)	.03‡
Adenoma-NOS	6 (6)	8 (6)	
Tubulovillous	10 (10)	17 (13)	
Villous	7 (7)	1 (1)	
Carcinoma in-situ	3 (3)	0 (0)	
Tubular adenoma, high-grade dysplasia	0 (0)	1 (1)	
Largest polyp ≥1 cm, No. (%)	25 (25)	40 (32)	.23
Treatment for prior polyp, No. (%)			
Complete endoscopic removal	92 (90)	117 (93)	.47
Surgery	10 (10)	9 (7)	
Baseline tissue polyamine content,‖ median (range), nmol/mg protein			
Putrescine	0.47 (0.01–4.60)	0.56 (0.01–5.29)	.48‡
Spermidine	1.99 (0.76–9.18)	2.17 (1.05–8.97)	.08‡
Spermine	6.82 (2.29–19.86)	7.29 (2.72–22.85)	.23‡
Spermidine to spermine ratio	0.30 (0.19–0.98)	0.31 (0.19–0.76)	.23‡

* N = 228; NOS = not otherwise specified.

† *P* value for the χ^2 test is listed unless noted otherwise. All statistical tests were two-sided.

‡ *P* value for the Wilcoxon rank sums test.

§ *P* value for the Fisher exact test.

‖ Tissue polyamine data missing for 1 subject with *ODC1* GG genotype and 1 subject with *ODC1* AA/GA genotype.

genotype, and interaction between treatment and genotype were examined using log binomial models as above but lacking gender and race in the model. Statistical analyses were conducted using SAS 9.2 statistical software (SAS, Inc, Cary, NC). All statistical tests were two-sided. Patients signed informed consent for trial inclusion and specimen retrieval and analysis. The study was approved by the University of California Irvine Institutional Review Board and by each institutional review board at participating study sites.

ODC1 genotype distribution was 126 GG (55%), 87 GA (38%), and 15 AA (7%). Patients in the two genotype groups differed in several baseline clinical characteristics (age, race, prior polyp histology; Table 1). *ODC1* genotype was not statistically significantly associated with a tissue putrescine response or spermidine to spermine ratio response in the full regression models (data not shown). The relative risk for adenoma recurrence related to treatment after adjustment in the full regression model was 0.39 (95% confidence interval = 0.24 to 0.66). A statistically significant interaction was detected between *ODC1* genotype and treatment in this model with respect to adenoma recurrence (placebo group: GG, 50%, AA/GA: 34%; treatment group: GG, 11%, AA/GA, 21%; $P_{\text{interaction}}$ = .038; Table 2). There were no statistically significant associations between treatment and *ODC1* genotype group with regard to cardiovascular or gastrointestinal adverse events (Table 2). No associations of treatment with ototoxicity were observed for *ODC1* genotype using the dominant

Table 2. Incidence of events after random assignment and stratified by *ODC1* genotype (dominant model)

Adverse events	Placebo (n = 111)		Eflornithine/sulindac (n = 117)		*P**
	GG, No. (%)	GA or AA, No. (%)	GG, No. (%)	GA or AA, No. (%)	
Any adenoma recurrence	22 (50)	18 (34)	7 (11)	9 (21)	<.001
Any adverse event					
Cardiovascular events†	8 (15)	8 (14)	13 (18)	9 (20)	.30
Gastrointestinal events‡	4 (7)	8 (14)	9 (13)	7 (15)	.54
Hearing loss of at least 15dB at ≥ 2 frequencies	10 (23)	9 (17)	14 (22)	11 (27	.26

* *P* value for the likelihood ratio test for treatment effect (eflornithine and sulindac vs placebo) on adenoma recurrence in the full model, which includes age (years, as a continuous variable), sex, race or ethnicity, aspirin use, treatment, genotype, and treatment and genotype interaction as covariates. A statistically significant interaction was detected in the full model for adenoma recurrence ($P = .038$); no interaction was detected for cardiovascular toxicity, gastrointestinal toxicity, or ototoxicity. All statistical tests were two-sided.

† Cardiovascular events included coronary artery disease, myocardial infarction, cerebrovascular accident, congestive heart failure, and chest pain.

‡ Gastrointestinal events included gastrointestinal bleeding (from any region), such as rectal bleeding, upper gastrointestinal bleeding, hematochezia, or occult blood in the stool.

model ($P = .26$; Table 2). Under a log-additive model, *ODC1* genotype was significantly associated with increased ototoxicity in the treatment arm ($P = .015$). Among patients receiving placebo or treatment, ototoxicity occurred in 23% vs 22% of *ODC1* GG patients, 20% vs 21% of *ODC1* GA patients, and 0% (zero of seven) vs 57% (four of seven) of *ODC1* AA patients, respectively. However, a test for interaction of genotype and treatment on ototoxicity was not statistically significant ($P = .45$).

Here, we observed that the adenoma inhibitory effect of eflornithine and sulindac was greater among those with the major G homozygous *ODC1* genotype, in contrast to previous reports showing decreased risk of recurrent adenoma among colorectal adenoma patients receiving aspirin and carrying at least one A allele (3–5). *ODC1* genotype distribution was similar to that reported in previous aspirin-based trials (3–5), and the A allele was associated with a non-statistically significant lower recurrent adenoma risk in the placebo group, consistent with previous reports (3,5). It is possible that the previously observed interaction with the *ODC1* A allele and aspirin may reflect a mechanism unique to aspirin or that alternate mechanisms are involved when nonsteroidal anti-inflammatory drugs are combined with an ODC inhibitor.

Study limitations include small sample size and a resultant limited number of events, as well as the lack of balance in baseline characteristics across *ODC1* genotype groups. It is acknowledged that sulindac and its metabolites have polyamine inhibitory properties (12) as well as other antineoplastic mechanisms, such as cyclooxygenase inhibition (13) and β-catenin degradation (14), which were not accounted for and could underlie our findings.

These results demonstrate that *ODC1* A allele carriers differ in response to prolonged exposure with eflornithine and sulindac compared with GG genotype patients. *ODC1* A allele carriers experience less treatment-related benefit (ie, metachronous adenoma risk reduction) and potential for higher risk of developing ototoxicity, especially among the AA homozygotes. Whether the A allele is a risk or protective allele may, therefore, depend on the tissue context or extent of polyamine inhibition. A major impediment to the translation of cancer chemoprevention research into clinical practice has been marginal agent efficacy and toxicities that exceed benefit (15,16). In this study, we identify genetic features that may be markers for both treatment benefit and toxicity. These results encourage evaluation in future polyamine inhibitory chemoprevention trials, as planned in the cooperative group setting (17).

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Funding

F.L.M. and E.W.G. have ownership interest in Cancer Prevention Pharmaceuticals, LLC. This work was supported in part by National Cancer Institute contract N01-CN75019 (F.L.M. and C.E.M.) and grants CA59024 (F.L.M.), CA88078 (F.L.M. and C.E.M.), P30 CA62230 (F.L.M.), CA23074 (E.W.G.), and CA95060 (E.W.G.).

Notes

Study design and analytic plan were developed by all coauthors. Data analyses were performed by W.-P. Chen and C. E. McLaren. All authors contributed to the writing and final approval of the article. The funders did not have any involvement in the design of the study; the collection, analysis, and interpretation of the data; the writing of the article; or the decision to submit the article for publication. The authors (J. A. Zell, C. E. McLaren, P. A. Thompson, E. W. Gerner, F. L. Meyskens) have a pending patent application related to concepts described in this article.

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The authors thank the clinical and research staff, Angela Garcia, Rachel Gonzalez, Yuhong Huang, Westley Lagerberg, Sharon Maxwell, and Lu Wu for their invaluable assistance with the study.

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